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[1]曹成,魏梅生,吴兴泉,等.菜豆荚斑驳病毒RT-PCR扩增产物胶体金层析检测方法的建立[J].大豆科学,2010,29(06):1024-1027.[doi:10.11861/j.issn.1000-9841.2010.06.1024]
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菜豆荚斑驳病毒RT-PCR扩增产物胶体金层析检测方法的建立

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关键词： 胶体金（KeySearch.aspx?type=KeyWord&Sel=胶体金）； 试纸条（KeySearch.aspx?type=KeyWord&Sel=试纸条）； 菜豆荚斑驳病毒（KeySearch.aspx?type=KeyWord&Sel=菜豆荚斑驳病毒）； RT-PCR（KeySearch.aspx?type=KeyWord&Sel=RT-PCR）； 核酸检测（KeySearch.aspx?type=KeyWord&Sel=核酸检测）

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摘要： 菜豆荚斑驳病毒是我国进境植物检疫性有害生物，为了能在现场快速检测出该病毒，将PCR核酸扩增的高灵敏度和胶体金层析方法快速简便、直观的优点结合起来，建立了PCR扩增产物的胶体金层析检测方法。该方法是用生物素标记上一个引物，用荧光素(或地高辛)标记上另一个引物，通过PCR扩增产生双标记的扩增产物。将兔抗生物素多克隆抗体与20～30 nm大小的胶体金颗粒结合上并固定在释放垫上，同时在硝酸纤维素层析膜上点上抗荧光素(或地高辛)的单克隆抗体作为T检测点，点上羊抗兔多克隆抗体作为C质控点。这种RT-PCR扩增产物可在T点上被检测到，同时C点也要显色。用所建立方法在15 min内可检测出菜豆荚斑驳病毒的RT-PCR扩增产物,免去了溴化乙锭染色和电泳的过程。

Abstract: Bean pod mottle virus (BPMV) is a quarantine pest for China. In order to detect the virus on site, we have developed a rapid method for detection of RT-PCR amplicon of the virus. The method is performed by labeling one prime with biotin the other primer with fluorescein (or digoxigenin). The dual-labeled amplicon can be generated from routine polymerase chain reaction. Rabbit polyclonal antibody against biotin is conjugated with 20~30 nm colloidal gold particles fixed in a conjugate pad. Monoclonal antibody against fluorescein (or digoxigenin) is spotted on nitrocellulose membrane as T dot. Goat anti-rabbit polyclonal antibody is spotted on nitrocellulose membrane as C dot. The RT-PCR amplicon is detected on the test dot(T dot), while the C dot serves as a control. Using this method we have detected the RT-PCR amplicon of bean pod mottle virus within 15 minutes without the staining of ethidium bromide and the running of agarose gel.

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